

## CLAIM LISTING

Claims 1, 4-6, 11 and 13 have been amended. Claims 2-3 and 10 have been canceled. Claims 1, 4-6, 11, 13 and 14 are now pending. This listing of claims will replace all prior versions and listings of claims in the Application.

### Listing of Claims:

#### WHAT IS CLAIMED IS:

Claim 1 (currently amended): A method for determining whether a human subject is at risk for developing obesity comprising ~~the steps of:~~

~~obtaining~~ assaying a sample from a human subject, said sample comprising a TBC1D1-encoding nucleic acid molecule or a complement thereof, ~~and to determine if there is detecting~~ an alteration in said TBC1D1-encoding nucleic acid molecule or said complement, said alteration being a cytidine to thymidine transition at the 373<sup>rd</sup> nucleotide of the TCB1D1 coding sequence of SEQ ID NO:1;

wherein the presence of said alteration identifies a subject at risk for developing obesity.

Claims 2-3 (canceled)

Claim 4 (currently amended): The method of Claim ~~[[3]]~~ 1 wherein said ~~detection~~ assaying step is conducted on genomic DNA.

Claim 5 (currently amended): The method of Claim ~~[[3]]~~ 1 wherein said ~~detection~~ assaying step is conducted on mRNA.

Claim 6 (currently amended): The method of Claim ~~[[2]]~~ 1, wherein said ~~nucleotide variant is detected by a method selected from the group consisting of~~ assaying step comprises at least one of the followings:

- a) hybridizing a probe specific for said alteration to RNA isolated from said human sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said alteration in the sample;
- b) hybridizing a probe specific for said alteration to cDNA made from RNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said alteration in the sample;
- c) hybridizing a probe specific for said alteration to genomic DNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said alteration in the sample;
- d) amplifying all or part of said TBC1D1-encoding nucleic acid molecule, or complement thereof, in said sample using a set of primers to produce amplified nucleic acids and sequencing the amplified nucleic acids;
- e) amplifying part of said TBC1D1-encoding nucleic acid molecule, or complement thereof, in said sample using a primer specific for said alteration and detecting the presence of an amplified product, wherein the presence of said product indicates the presence of said alteration in the sample;
- f) molecularly cloning all or part of said TBC1D1-encoding nucleic acid molecule, or complement thereof, in said sample to produce a cloned nucleic acid and sequencing the cloned nucleic acid;
- g) amplifying said TBC1D1-encoding nucleic acid molecule, or complement thereof, to produce amplified nucleic acids, hybridizing the amplified nucleic acids to a DNA probe specific for said alteration and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said alteration;
- h) forming single-stranded DNA from a gene fragment of said TBC1D1-encoding nucleic acid molecule, or complement thereof, from said human sample and single-stranded DNA from a corresponding fragment of a wild-type gene, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to wild-type and sequencing said single-stranded DNA having a shift in mobility;

i) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said sample and a second strand of a nucleic acid consisting of a corresponding human wild-type gene fragment, analyzing for the presence of a mismatch in said heteroduplex, and sequencing said first strand of nucleic acid having a mismatch;

j) forming single-stranded DNA from said TBC1D1-encoding nucleic acid molecule, or complement thereof, of said human sample and from a corresponding fragment of an allele specific for said alteration, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to said allele, wherein no shift in electrophoretic mobility of the single-stranded DNA relative to the allele indicates the presence of said alteration in said sample; and

k) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment of said TBC1D1-encoding nucleic acid molecule, or complement thereof, isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said sample and a second strand of a nucleic acid consisting of a corresponding gene allele fragment specific for said alteration and analyzing for the presence of a mismatch in said heteroduplex, wherein no mismatch indicates the presence of said alteration.

Claim 7 (withdrawn): The method of Claim 1 wherein said detection step comprises detecting the presence or absence of an amino acid substitution in said TBC1D1 protein.

Claim 8 (withdrawn): The method of claim 7 wherein said alteration is an amino acid substitution selected from the group consisting of R125W, V228G or L392V in a TBC1D1 protein.

Claim 9 (withdrawn): The method of Claim 8 wherein said amino acid substitution is detected by a method selected from the group consisting of:

- (a) immunoblotting;
- (b) immunocytochemistry;
- (c) enzyme-linked immunosorbant or immunofiltration assay; or
- (c) assaying the affinity of binding between said TBC1D1 protein and phosphotyrosine, or a peptide containing a phosphotyrosine residue.

Claim 10 (canceled).

Claim 11 (currently amended): The method of claim ~~[[2]]~~ 1, wherein said ~~detection~~ assaying step comprises hybridizing a nucleic acid probe specifically hybridizable to an altered TBC1D1 coding sequence, or complement thereof.

Claim 12 (withdrawn): The method of claim 1 comprising the steps of:

- (a) contacting an antibody capable of binding a polypeptide comprising an altered TBC1D1 amino acid sequence but incapable of binding an analogous wild-type TBC1D1 polypeptide; and
- (b) detecting binding of said antibody to said altered TBC1D1 polypeptide or lack of binding to said wild-type TBC1D1 polypeptide.

Claim 13 (currently amended): A method for predicting, in a human subject, the likelihood of developing obesity associated with genetic variants of the human *TBC1D1* gene comprising detecting the presence or absence of:

- ~~a cytidine to thymidine transition at the 466<sup>th</sup> nucleotide in the sense strand of the first TCB1D1 coding exon, ~~[[()]]~~SEQ ID NO:33), or the complement thereof;~~
- a cytidine to thymidine transition at the 373<sup>rd</sup> nucleotide of the ~~TCB1D1~~ TBC1D1 coding sequence of SEQ ID NO:1, ~~or the complement thereof;~~
- ~~a cytidine to thymidine transition at the 373<sup>rd</sup> nucleotide of the TCB1D1 coding sequence of an alternative transcript comprising the coding sequence encoded by the first TCB1D1 coding exon (SEQ ID NO:33), or the complement thereof;~~
- ~~or a nucleotide variant that results in an arginine to tryptophan substitution at the 125<sup>th</sup> amino acid residue of a TBC1D1 protein, or the complement thereof;~~

in a *TBC1D1* encoding nucleic acid of said subject;  
wherein the presence of said nucleotide variant predicts that said subject has an increased likelihood of developing obesity.

Claim 14 (original): The method of claim 13, wherein said nucleotide variant associated with obesity is detected by determining the genomic sequence of said *TBC1D1* gene.

Claim 15 (withdrawn): A method of screening for drug candidates useful in treating obesity comprising:

- (a) preparing an assay solution comprising *TBC1D1*, or a homolog, derivative, or fragment thereof,
- (b) measuring the level of biological activity of said *TBC1D1*, or a homolog, derivative, or fragment thereof in the presence and absence of a test compound, and
- (c) detecting a difference in said biological activity in the presence or absence of said test compound;

wherein a detected difference in said biological activity in the presence and absence of said test compound indicates that said test compound is a drug candidate.

Claim 16 (withdrawn): The method of Claim 15, wherein said biological activity is the binding of phosphotyrosine, or a phosphotyrosine-containing peptide, by *TBC1D1*.

Claim 17 (withdrawn): The method of Claim 15, wherein said biological activity is the ability to form protein:protein interactions.

Claim 18 (withdrawn): The method of Claim 15, wherein said *TBC1D1*, or the homolog, derivative, or fragment thereof, is an altered form thereof, bearing an amino acid substitution.

Claim 19 (withdrawn): The method of Claim 18, wherein said altered form bears an amino acid substitution, relative to SEQ ID NO:2, selected from the group consisting of R125W, V228G or L392V.

Claim 20 (withdrawn): The method of claim 15 further comprising testing said drug candidate in cell or animal obesity disease model.